0845 – 0915 Fundamentals of cannabinoid neuroscience

PHARMACOLOGICAL STRATEGIES FOR TARGETING CANNABINOID RECEPTORS IN THE CLINIC

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It is now generally accepted that the endocannabinoid system adopts an "autoprotective" role in certain disorders. This discovery has provided additional rationale for the approved medical applications of cannabinoid receptor agonists such as delta-9-tetrahydrocannabinol and encouraged a search for new therapeutic uses for these agonists. It has also led on to the identification of several potential strategies for activating the endocannabinoid system in the clinic with improved selectivity. These include "direct" strategies that rely on the selective activation of (i) cannabinoid CB_2 receptors or (ii) cannabinoid CB_1/CB_2 receptors that are located outside the blood-brain barrier or are expressed by a particular tissue. They also include "indirect" strategies that rely on the ability of an administered compound to increase cannabinoid receptor activation by endogenously released endocannabinoids when these are inducing sought-after effects. These are strategies that involve (i) increasing endogenous levels of the endocannabinoids, anandamide and/or 2-arachidonoylglycerol, by inhibiting their cellular uptake or intracellular metabolism or (ii) increasing endocannabinoid-induced activation of the CB_1 receptor through allosteric enhancement. One other potential strategy is "multi-targeting". Thus, there have been reports that certain cannabinoid receptor agonists can interact in an additive or synergistic manner at relatively low doses either with a non-cannabinoid or, intriguingly, with a cannabinoid receptor antagonist, to reduce unwanted symptoms such as pain, anxiety, depression and emesis. There is also evidence that simultaneous blockade of CB_1 receptors and activation of CB_2 receptors may ameliorate pain, Parkinson's disease, myocardial infarction, stroke and chronic liver diseases. Importantly, therefore, the plant cannabinoid, Δ^9-tetrahydrocannabivarin (Δ^9-THCV), has recently been discovered to block CB_1 receptors but activate CB_2 receptors and to possess both anti-inflammatory and analgesic properties. Mention will also be made of the extent to which certain well-known CB_1 and/or CB_2 receptor ligands, including delta-9-tetrahydrocannabinol, interact with other kinds of receptor, for example GPR55.

ENDOCANNABINOIDS AND THE CONTROL OF PAIN INITIATION

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Endocannabinoids, the brains own cannabis-like substances, are mobilized on demand to produce adaptive changes in pain responsiveness. Monoacylglycerol lipase (MGL) and fatty acid amide hydrolase (FAAH) degrade the endocannabinoids 2-arachidonoylglycerol (2-AG) and anandamide (AEA), respectively. Our previous work used an animal model of stress-induced analgesia to identify the enzyme monoacylglycerol lipase (MGL) as a previously unrecognized therapeutic target for pain. The MGL-preferring inhibitor URB602, administered locally in the periaqueductal gray (PAG), enhanced the antinociceptive effects of stress through a cannabinoid CB_1-dependent mechanism. The same behaviorally active dose of URB602 also elevated levels of endogenous 2-AG without altering levels of anandamide. Selective inhibition of MGL and FAAH in the periphery may, thus, elucidate the role of endocannabinoids in controlling pain initiation in animal models of persistent pain. We compared peripheral antinociceptive effects of a novel selective MGL inhibitor, JZL184, with the MGL-preferring inhibitor URB602 and exogenous 2-AG. Intra-paw administration of JZL184 and URB602 suppressed both early and late phases of formalin pain. Both MGL inhibitors produced additive antinociceptive effects when combined with exogenous 2-AG. The dose response curves for each MGL inhibitor had the same slope, suggesting that JZL184 and URB602 acted through a common mechanism, albeit with different potencies. Antinociceptive effects of both MGL inhibitors were also blocked by either a CB1 or CB2 antagonist. Based upon these observations, we compared peripheral antinociceptive effects of the MGL inhibitor JZL184, the FAAH inhibitor URB597, and the endocannabinoid uptake inhibitor VDM11, on hypersensitivities produced by capsaicin, the pungent ingredient in hot chili peppers. Intradermal capsaicin produced nocifensive behavior, thermal hyperalgesia, and mechanical allodynia in rats. JZL184 suppressed capsaicin-induced nocifensive behavior and thermal hyperalgesia but did not alter capsaicin-evoked mechanical allodynia. As in the formalin model, effects of JZL184 were blocked by either a CB1 or a CB2 antagonist. By contrast, the FAAH inhibitor URB597 suppressed capsaicin-induced mechanical allodynia without altering capsaicin-evoked thermal hyperalgesia or nocifensive behavior. Effects of URB597 were blocked by a CB1 antagonist but not by a CB2 antagonist. Finally, VDM11, a putative inhibitor of endocannabinoid uptake, suppressed capsaicin-evoked hypersensitivity for all three dependent measures (nocifensive behavior, thermal hyperalgesia, and mechanical allodynia), suggesting an additive effect following putative elevation of both AEA and 2-AG. The antinociceptive effects of VDM11 exhibited a pattern of pharmacological specificity that was mimicked by that of the MGL and FAAH inhibitor in combination. Thus, peripheral inhibition of MGL and FAAH suppresses capsaicin-evoked behavioral sensitization with distinct patterns of pharmacological specificity and in a non-overlapping and modality-specific manner. Modulation of endocannabinoids in the periphery suppressed capsaicin-evoked nocifensive behavior and thermal hyperalgesia through either CB1 or CB2 receptor mechanisms but suppressed capsaicin-evoked mechanical allodynia through CB1 mechanisms only. Our studies also suggest that inhibition of endocannabinoid transport was more effective in suppressing capsaicin-induced sensitization compared to inhibition of either FAAH or MGL alone. More work is necessary to validate the efficacy of inhibitors of endocannabinoid deactivation and uptake as analgesics.
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OPIOIDS AND CANNABINOIDS INTERACTIONS: INVOLVEMENT IN PAIN MANAGEMENT

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Among several pharmacological properties, analgesia is the most common feature shared by either opioid or cannabinoid systems. Cannabinoids and opioids are distinct drug classes that have been historically used separately or in combination to treat different pain states. Indeed, it is widely known that activation of either opioid or cannabinoid systems produce antinociceptive properties in different pain models. Moreover, several biochemical, molecular and pharmacological studies support the existence of reciprocal interactions between both systems, suggesting a common underlying mechanism. Further studies have demonstrated that the endogenous opioid system could be involved in cannabinoid antinociception and recent data have also provided evidence for a role of the endogenous cannabinoid system in opioid antinociception. These interactions may lead to additive or even synergistic antinociceptive effects, emphasizing their clinical relevance in humans in order to enhance analgesic effects with lower doses and consequently fewer undesirable side effects. Thus, this presentation is focused on bidirectional interactions between opioids and cannabinoids and their potent repercussions on pain modulation.

1045 – 1115 Cannabinoids and comorbidity

CANNABINOIDS AND DEPRESSION

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Accumulating evidence has implicated deficient endocannabinoid signaling in the etiology of depression. Endocannabinoid signaling is found to be reduced in both preclinical models of depression and in clinical populations diagnosed with major depression. Furthermore, deficits in endocannabinoid signaling produce a phenotype reminiscent of many of the symptoms of mood disorders, such as impaired reward sensitivity, heightened emotional reactivity, alterations in cognitive and neurovegetative functions and increased HPA axis activity. Interestingly, facilitation of endocannabinoid signaling produces many of the biochemical and behavioral signatures of conventional antidepressant agents, such as facilitating serotonergic neurotransmission, enhancing cellular resilience within the brain, dampening psychoneuroendocrine responses to stress as well as producing both anti-inflammatory and analagesic effects. These combined preclinical and clinical findings paint an intriguing picture in which the endocannabinoid system may play a central role in the genesis or maintenance of mood disorders. In line with this hypothesis, these data also support the rationale for the clinical investigation of agents which inhibit the cellular uptake and/or metabolism of endocannabinoids in the treatment of mood disorders.

CANNABINOID RECEPTOR-TRP CHANNEL INTERACTIONS IN PAIN AND INFLAMMATION

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The transient receptor potential (TRP) channels of the vanilloid-type 1-4 (TRPV1-4), ankyrin type-1 (TRPA1) or melastatin type-8 (TRPM8) are involved in thermosensation, pain transduction and inflammation. They are expressed in sensory fibers of Aδ and C-type, in dorsal root and trigeminal ganglia as well as in perivascular neurons, with TRPV1 (the "capsaicin receptor") and TRPA1 (the "mustard receptor") being often co-expressed in the same neurons. Whilst TRPV1-4 are activated by high temperatures, TRPA1 and TRPM8 (the "menthol receptor") are activated by cold. TRPV1 is also activated by low pH, such as during certain inflammatory conditions, as well as by several pro-inflammatory mediators, and this leads to release of vasodilatory peptides from sensory neurons, thus contributing to neurogenic inflammation. TRPA1, instead, is activated by numerous irritants. TRPV1 is also expressed in central neurons. It is abundant in the periaqueductal grey (PAG) and rostral ventrolateral medulla (RVM), where it modulates the descending pathway of antinociception. Contrary to its role in the spinal cord and sensory afferents, TRPV1 in the PAG-RVM contributes to descending antinociception by enhancing both glutamatergic signalling/OFF neuron activity in the RVM and μ-opioid receptor-mediated analgesia. TRPV1 is expressed in the hippocampus, prefrontal cortex and amygdala, where it may play a role in fear and anxiety.

In both central and sensory neurons, TRPV1 is often co-expressed with cannabinoid CB_1 receptors, with which it shares two endogenous agonists, anandamide and *N*-arachidonoyl-dopamine (NADA). TRPV1 and CB_1 can either act in concert or oppose each other at modulating neurotransmitter release. Furthermore, several plant cannabinoids, such as THC, cannabidiol or cannabichromene, activate at least one among TRPV1, TRPV2 and TRPA1 channels, and/or potently antagonize TRPM8 channels. Importantly, CBD can both activate and desensitise TRPV1, TRPV2 and TRPA1 channels, and this property endows the compound with the capability of potentially influencing nociception and inflammation in several ways. These interactions, together with the finding of an increasing number of physiopathological functions that anandamide exerts via TRPV1 channels, suggest that TRP channels are true "ionotropic cannabinoid receptors".

CANNABINOIDS IN CANCER PAIN

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Cannabinoids, a family of compounds isolated from the *Cannabis sativa* plant (marijuana), have been increasingly used in several areas of medicine, including the management of pain. Several clinical trials have shown the benefit of cannabinoids in a variety of pain states. Cancer pain has been investigated in the past as a possible target area (especially with regard to neuropathic pain), with mixed results. However, the development of newer compounds and delivery options have brought about a resurgence in clinical trials for cancer pain. Preclinical studies support a role in the treatment of chemotherapy-induced neuropathy as well as a possible role in neuroprotection. Early evidence also points to potential uses as an anti-cancer agent, which in turn may result in a less cancer pain. A review of the literature and some early clinical research findings will be presented, with the focus on those results that may hold promise for cancer patients.

1330 – 1400 Cannabinoids in clinical practice

TAPPING INTO THE ENDOCANNABINOID SYSTEM TO ALLEVIATE OSTEOARTHRITIS PAIN

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We have recently shown that knee joints possess cannabinoid-1 (CB1) receptors and local activation of these receptors with a selective CB1 agonist reduces nociceptor activity in a model of osteoarthritis (OA)[1]. Recently, we examined whether enhancement of endogenous cannabinoid levels by peripheral and systemic administration of the fatty acid amide hydrolase inhibitor URB597 could modulate OA pain.

In male Wistar rats, OA was induced by injecting 3mg of sodium monoiodoacetate (MIA) into the knee joint and allowing animals to recover for 14 days. In another model, Dunkin-Hartley guinea pigs (age 9-12 months) develop OA naturally and were used as a model of spontaneous OA. Joint nociception was objectively measured in these animals by recording electrophysiologically from knee joint primary afferents in response to normal rotation and noxious hyper-rotation of the joint both before and following close intra-arterial injection of URB597 (30ug;100ul bolus). The effect of systemic URB597 administration (5mg/kg) on joint pain perception in the MIA model was determined by hindlimb weight bearing.

Local injection of URB597 reduced the mechanical sensitivity of joint pain sensing nerve fibres in OA rats and guinea pigs with nerve firing rate decreasing by about 60%. Systemic URB597 administration significantly reduced hindlimb incapacitance in OA rats. These anti-nociceptive and analgesic effects of URB597 could be blocked by pre-administration of the CB1 receptor antagonist AM251 but not the CB2 receptor antagonist AM630.

Our data demonstrate the utility of inhibiting fatty acid amide hydrolase activity to enable the build up of endocannabinoid levels in the joint leading to a reduction in OA pain. Thus, targeting the peripheral endocannabinoid system could be a viable means of alleviating OA pain for millions of patients.

1.Schuelert, N. & McDougall, J.J. Cannabinoid-mediated antinociception is enhanced in rat osteoarthritic knees. *Arthritis Rheum.* **58**, 145-153 (2008).

1400 – 1430 Cannabinoids in clinical practice

CANNABINOIDS IN HIV/AIDS

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The use of medical marijuana for the treatment of HIV/AIDS is explored. A review of background pharmacology of cannabinoids is presented. Clinical trials, including the use of cannabinoids for the treatment of AIDS wasting and HIV neuropathy are discussed.

PERIPHERALLY-ACTING CB1-CB2 AGONISTS FOR PAIN: DO THEY STILL HOLD PROMISE?

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There is a large body of pre-clinical evidence supporting a peripherally-mediated analgesic action of cannabinoids. We hypothesized that their therapeutic window could be improved, notably vs. their central side-effects, by limiting their access to the central compartment. To test the hypothesized peripheral analgesic mechanism and to assess the optimal level of peripheral restriction to combine efficacy with acceptable tolerability profile, we initiated clinical studies with two novel orally active mixed CB1/CB2 agonists (AZD1940 & AZD1704) characterized by different extent of brain uptake (rat Cbr/Cpl ratio of ~0.1 & 0.01 for AZD1940 & AZD1704 respectively) and different CNS psychoactivity in a rat Δ9-THC drug discrimination test. Both compounds were orally active in various rat models of nociceptive and neuropathic pain and displayed improved safety margins vs. central side effects typically observed with known CB1 agonists. No pre-clinical safety issues preventing initiation of clinical studies were identified. In clinical phase I single ascending dose (SAD) study, AZD1940 maximum tolerated dose (MTD) was 0.8 mg with plasma exposure of 1.7 nM free. The clinical efficacy of AZD1940 as a pain relief agent was explored in two single dose phase II studies (human capsaicin and 3rd molar extraction models) and in the multiple ascending dose (MAD) study performed with volunteers affected by chronic low back pain. The 2 single dose phase II studies conducted with the MTD showed no effects on primary endpoints (pain intensity and heat pain threshold for capsaicin study). In the multiple ascending dose (MAD) study where AZD1940 was administered for 12 days, repeated dosing led to slow compound accumulation ($t_{1/2}$ ~ 80h). A daily 1mg dose led to a plasma exposure at steady state of 7 nM free. Significant weight gain and some hepatic enzymes increase were noticed. During the SAD study, AZD1704 exhibited hypotensive effects (up to 20 mm Hg supine systolic blood pressure drop with 2.4mg oral dose corresponding to a plasma exposure of ~2.0 nM free). No CNS adverse-events were noticed. The measured $t_{1/2}$ (6-8h) was too short to consider repeated dosing to investigate potential tolerance development to the blood pressure lowering effects. To conclude, CB1 agonists with limited CNS access have been observed to display a different tolerability profile in human compared to the more brain-permeable CB1 agonists. After our limited studies, optimal clinical efficacy of peripherally restricted CB1 agonists remains to be proven. In addition, it is unclear how the hemodynamic and metaboli effects observed in these clinical studies could be completely managed for this class of cannabinoids.

SELECTIVE FATTY ACID AMIDE INHIBITION FOR THE TREATMENT OF CHRONIC PAIN

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Pfizer Worldwide R&D

Fatty acid amide hydrolase (FAAH) is an integral membrane serine hydrolase that degrades the fatty acid amide family of signalling lipids, including the endocannabinoid anandamide. Genetic or pharmacological inactivation of FAAH leads to analgesic and anti-inflammatory phenotypes in rodents without showing the undesirable side effects observed with direct cannabinoid receptor agonists, indicating that FAAH may represent an attractive therapeutic target for the treatment of inflammatory pain. This talk will briefly highlight the discovery and characterization of our irreversible piperidine urea FAAH inhibitor PF-04457845. This compound covalently modifies the active-site serine nucleophile of FAAH with exquisite selectivity relative to other members of the serine hydrolase superfamily. PF-04457845 demonstrates an attractive analgesic profile in preclinical models of inflammatory pain with efficacy comparable to NSAIDS. Furthermore, analgesia is clearly correlated with FAAH inhibition and fatty acid amide elevation measured ex vivo in brain and plasma. PF-04457845 represents an exciting candidate to explore the effects of FAAH inhibition in patients and has been progressed to clinical studies in chronic pain.

SATIVEX®, AND THE PHYTOCANNABINOID PIPELINE FOR PAIN CONTROL

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Cannabinoids alleviate pain via direct analgesic and anti-inflammatory effects, modulation of neurotransmitters, and interactions with opioids. Cannabinoids of botanical origin may be approvable by FDA following dictates of their *Botanical Guidance.*

Sativex® is a whole-cannabis-based extract produced by GW Pharmaceuticals in the UK, delivered as an oromucosal spray combining a CB_1 and CB_2 partial agonist (THC) with a cannabinoid system modulator (CBD), minor cannabinoids and terpenoids plus ethanol and propylene glycol excipients and peppermint flavoring. Sativex is approved for prescription in the UK and Spain for spasticity in multiple sclerosis, and in Canada for central neuropathic pain in MS and cancer pain unresponsive to opioids. Sativex is highly standardized, and formulated from two *Cannabis sativa* chemotypes predominant in THC and CBD respectively. Each 100 µL pump-action spray of Sativex yields 2.7 mg of THC and 2.5 mg of CBD plus additional components. Sativex effects begin in an intermediate time, allowing dose titration. Most patients stabilize at 8-10 sprays per day within one week, attaining symptomatic control without undue adverse events (AE). Sativex has been added to optimized drug regimens in subjects with uncontrolled pain in numerous RCTs. An Investigational New Drug (IND) application is active in the USA in patients with intractable cancer pain. Phase IIA and IIB studies have been successfully completed.

The AE profile of Sativex has been quite benign with bad taste, oral stinging, dry mouth, dizziness, nausea or fatigue most common, but not usually prompting discontinuation. Most psychoactive sequelae are early and transient, and have been notably improved by a slower titration schedule. Euphoria and reinforcing psychoactive indicia are uncommon. Sativex has not demonstrated dose tolerance to its therapeutic benefits on prolonged administration and efficacy has been maintained for up to several years in pain conditions. No known abuse or diversion incidents have been reported to date (August 2010). Formal Drug Abuse Liability studies of Sativex vs. Marinol and placebo demonstrate lower scores on drug liking and similar measures at comparable doses.

Additional phytocannabinoids show promise in treatment of chronic pain, including cannabichromene, cannabigerol, and tetrahydocannabinvarin. Chemotypes expressing high proportion of each are available and undergoing research in the GW development program. Cannabis terpenoid components also demonstrate analgesic activity, notably β-caryophyllene, a selective CB_2 agonist.

The unique pharmacological profile of Sativex with multimodality analgesic benefits and favorable efficacy and safety profiles render it and prospective phytocannabinoid pharmaceuticals promising agents for adjunctive treatment, particularly for neuropathic pain.